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Voltage-Gated Membrane Current of Isolated Bullfrog Taste Cells

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ABSTRACT—The membrane properties of enzymatically isolated bullfrog taste cells were studied using the whole-cell patch-clamp technique. Five voltage-dependent ionic currents were observed in the bullfrog taste cells: a transient tetrodotoxin (TTX)-sensitive Na^+ current; a Ca^{2+}-activated K^+ current; a transient K^+ current; a sustained K^+ current; and an inward rectifier K^+ current. The transient inward Na^+ current, Ca^{2+}-activated and transient outward K^+ currents began to be activated by depolarization to -40 to -20 mV from a holding potential more negative than -60 mV, while a novel transient outward current was activated by voltage steps to the potential more positive than $+40$ mV from a holding potential of -40 mV in Ca^{2+}-free saline. The sustained current was not inactivated at least 5 min after onset of voltage shift from -60 to -40 mV. We did not find any Ca^{2+} current under the condition of the present experiment. The inward rectifier K^+ current was observed in normal and high K^+ saline, and the amplitude and the reversal potential for the current seemed to change depending upon the external K^+ concentration. These results suggest that the inward currents produced by the inward rectifier K^+ channel as well as other K^+ channels contribute to gustatory transduction of KCl stimuli into the receptor potential if those channels are located in the receptive membrane.

INTRODUCTION

Taste cells are modified lingual epithelial cells that are responsible for transduction of chemical information on the tongue into electrical signal and for transmission of this signal to the gustatory nerves. Bullfrog taste cells lie in the taste disc, which is a modified form of the mammalian taste bud [1]. The taste cell plasma membrane is divided by tight junction into an apical surface exposed to lingual fluid (receptive membrane) and a basolateral surface bathed in interstitial fluid (basolateral membrane) [1, 2].

The membrane properties and responsse characteristics of taste cells to various taste stimuli have been examined in detail using conventional intracellular recording techniques (reviewed in Sato, 1980 [3]). The resting potential of bullfrog taste cells ranged from -20 to -30 mV in the average, and the current-voltage relationship of the cell membrane was linear. Action potential has never been observed in response to a depolarization from the resting potential, although anodal break spike potentials have been observed at the cessation of strong hyperpolarization [4]. No action potentials have been observed in taste cells of catfish and mouse by conventional intracellular recording method [5], while action potentials evoked by both electrical and gustatory stimulations have been reported in *in situ* taste cells of mudpuppy [6–8].

Recently, application of the patch-clamp method to amphibian taste cells has resulted in the detailed characterization of their membrane currents [9–11]. These studies indicate that the taste cell has a resting potential (zero-current potential) of approximately -60 mV, and a membrane resistance on the order of 1–2 GΩ, and displays voltage-dependent currents similar to those of neurons. These observations suggest that there are significant differences between the membrane properties in isolated and *in situ* taste cells.

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Little is known about voltage- or tastant-gated currents under voltage clamp condition in the bullfrog (*Rana catesbeiana*) taste cells, although characteristics of taste cell responses in *in situ* taste organ have extensively been studied using this animal [3, 4, 12–16]. In the present experiment, we studied the membrane currents evoked by electrical stimulation in enzymatically isolated bullfrog taste cells using the whole-cell patch-clamp technique, and we found one type of Na^+ current and several types of K^+ currents. The results suggest that the membrane properties of bullfrog taste cells are very similar to those of neurons, and some voltage-dependent channels play an important role in taste transduction.

MATERIALS AND METHODS

Cell isolation

Isolated taste cells were obtained from the tongue surface of decapitated and pithed adult bullfrogs (*Rana catesbeiana*) weighing 325–518 g. The fungiform papillae were removed from tongue in normal saline (see below). Cells in the papilae were dissociated by the same method as described before [17].

Solutions

Normal saline solution consisted of (in mM): NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Na-HEPES, 5; glucose, 20 (pH 7.2). TTX was added to normal saline but Co^{2+}, Ba^{2+}, 4-aminopyridine (4-AP) and tetraethylammonium (TEA) were substituted for Na^+ and Ca^{2+}. In Na^+-free saline and high K^+ saline solutions, Na^+ was replaced with *N*-methyl-D-glucamine$^+$ ($NMDG^+$) and K^+, respectively.

The pipette solution (K-Mg sol.) contained (in mM): KCl, 100; $MgCl_2$, 2; EGTA, 5; K-HEPES, 5 (pH 7.2). In later experiments, 2 mM Na_2ATP was added (K-Mg-ATP sol.) to prevent Ca^{2+} channel run-down [18]. In some experiments, KCl was replaced with CsCl (Cs-Mg-ATP sol.). Pipette solution not containing Mg^{2+} and ATP (Ksol.) was cometimes used.

Recording procedure

Voltage- and current-clamp experiments were performed using the standard whole-cell patch-clamp technique [19]. Patch pipettes were pulled from thick-walled glass capillaries containing a fine filament (outer diameter, 1.4 mm; Summit Medical, Tokyo, Japan) on a two-stage puller (Narishige PD-5, Tokyo, Japan). The tips of the electrodes were heat-polished with a microforge (Narishige MF-83, Tokyo, Japan). The resistance of the resulting patch electrodes was 3–10 MΩ when filled with internal solution. In some experiments, silicone (Shin-Etsu Silicone, KT-16-1000CS, Tokyo, Japan) was used to coat the tip of electrode to minimize capacitance transients. The recording was made from taste cells attached to coverslips at the bottom of chamber put on the stage of an invertmicroscope (Olympus IMT-2, Tokyo, Japan). The recording pipette was positioned with a hydraulic micromanipulator (Narishige MO-103N-L, Tokyo, Japan).

Whole-cell current or voltage was measured with a patch-clamp amplifier, EPC-7 (List-electronics, Darmstadt, FRG). The results were low-pass filtered at 1–3 kHz, stored on a magnetic tape (Teac R-210A, Tokyo, Japan), and digitized with a memory oscilloscope (Nihon Kohden VC-10, Tokyo, Japan). Initial sealing of the pipette to the cell surface was facilitated by applying weak suction (10 cm H_2O) after releasing a slight positive pressure. After compensating the capacitance and adjusting the holding voltage, the patch membrane was broken by applying strong suction, resulting in a sudden increase in capacitance to 5–20 pF. The indifferent electrode was a 3%-agar-normal saline electrods for most experiments, but a 3%-agar-3 M KCl for high K^+ experiments to eliminate changes in the indifferent electrode potential.

RESULTS

Morphology of taste cells

The morphology of the bullfrog taste cells has been investigated using electronmicroscopy [1, 2], and recently by intracellular dye injection [20].

The taste cells which renged from 40 to 70 μm (61.0 ± 1.5 μm, n=25) in length were bipolar type cells characterized by bifurcated or trifurcated branches of a large dendritic process [17]. The branches of 10–40 μm (25.4 ± 1.1 μm, n=25) in length and about 2 μm in diameter were connected with each other by a thin membranous structure. The taste cells had ellipsoidal cell bodies, ranged from 5 to 12 μm (8.5 ± 0.3 μm, n=25) in diameter. Opposite the dendritic processes, some cells gave rise to irregular stumpy processes of 2–5 μm in length and 2 μm in diameter. Typical cells averaged two stumpy processes, having a knob-like structure at the tip portion. All the taste cells studied here were identified on the basis of those characteristic features.

We observed at least three types of cells other than typical taste cells in the taste disc. A few bipolar cells lacking dendritic branches were encountered when vigorous trituration was performed to dissociate cells from taste discs. The size of the cell was approximately the same as that of the taste cell. The cell with vigorously beating cilia was observed. There were many round cells ranging from 5 to 20 μm in diameter [17]. We frequently observed that the tip of taste cell dendrite was attached to the surface of rund cells.

Whole-cell recording

Within a few minutes after obtaining the whole-cell configuration with K-Mg solution in the pipette, the zero-current potential shifted from an initial value of -59 ± 2 mV (n=34) to -75 ± 1 mV (n=26). Membrane resistance, which was measured by applying -10 mV voltage steps from a holding potential of -65 mV, varied with enzyme concentration employed for cell dissociation. The higher the concentration of enzyme, the lower the membrane resistance, but the easier gigaohm seal was obtained. At a low concentration of enzyme taste disc cells were not dissociated easily. Eventually, we used an appropriate concentration of enzyme which gave good dissociation and seals. The isolated taste cells had a membrane resistance of 1.6 ± 0.3 GΩ (n=26) and a membrane capacitance of 15 ± 1 pF (n=26) with a K-Mg pipette solution,

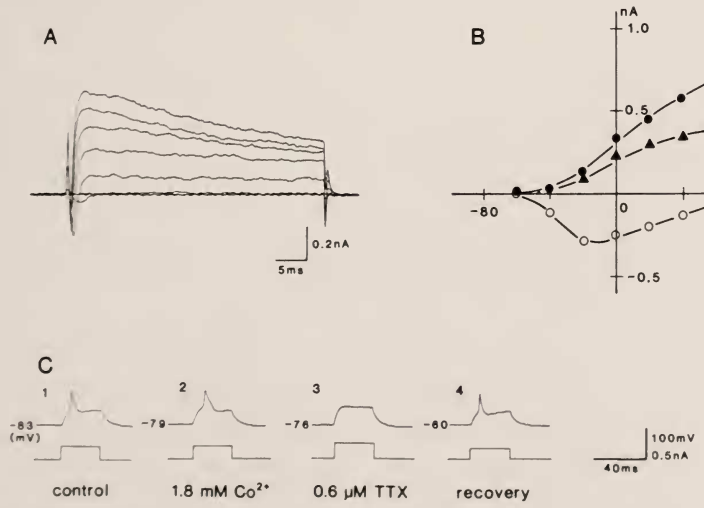

FIG. 1. Typical responses of a dissociated taste cell to electrical stimulation. A, voltage-clamp records. The taste cell was stimulated by 40 ms-voltage steps between -60 and 60 mV in 20 mV increment from a holding potential of -80 mV. B, I-V relationships of the peak inward current (○) and the peak (●) and steady state (▲) outward currents from the same cell in A. Holding potential was -80 mV, and the pipette contained K-Mg-sol. C, voltage responses (upper traces) of another cell to depolarizing current injections (lower traces). The falling phase of the action potential was slower in 1.8 mM Co^{2+} saline (C2) than in normal saline (C1), but the amplitude was not affected. The action potential was completely blocked by addition of 0.6 μM TTX (C3). Effects of the drugs were fully reversible as shown in reappearance of action potential in normal saline (C4).

whenever seal resistances greater than 10 GΩ were obtained.

When K^+ in the pipette solution was replaced with 100 mM Cs^+ (Cs-Mg-ATP sol.), the membrane resistance increased significantly (11.6±1.8 GΩ, n=16). Most of the taste cells responded to depolarizing voltage steps from a holding potential of −80 mV with an initial transient inward current followed by a delayed outward current (Fig. 1A, B). Under current clamp, these cells generated a single action potential in response to depolarizing current injection (Fig. 1C). Repetive spike potentials were never generated even after an injection of long depolarizing current. Three types of taste cells were distinguished on the basis of the ratio of inward to outward current magnitudes. The first type generated a large inward current with a maximum peak value of more than 1 nA, followed by a relatively small outward current. The second type showed intermediate inward and outward currents. The third type generated small or no inward current followed by a large outward current. The first and second type of taste cells discharged a single action potential in response to depolarizing current injection, but the third type of the cell was inexcitable in normal saline [17]. All analyses of currents described below were obtained from the second cell type.

Na^+ current

When K^+ in the pipette solution was replaced with Cs^+, nearly all outward current was blocked and only a transient inward current remained (Fig. 2A, B). The inward current was completely eliminated when $NMDG^+$ was substituted for Na^+ in the bathing solution (Fig. 2C). The action potential (Fig. 1C) and the corresponding early inward current (Fig. 2D) were completely blocked by 0.6 μM TTX in the bathing solution. The threshold of inward current activation was −30 to −20 mV at holding potential of approximately −60 mV (Figs. 2B, 4B).

Figure 3A shows a steady state inactivation curve for the transient inward current. The normalized peak currents generated by voltage steps to −15 mV from different holding potentials (see inset) are plotted against the holding potentials. Half the maximal inactivation occurred at −52±4 mV (n=3), and the inward current was completely inactivated between −40 and −20 mV. Figure 3B

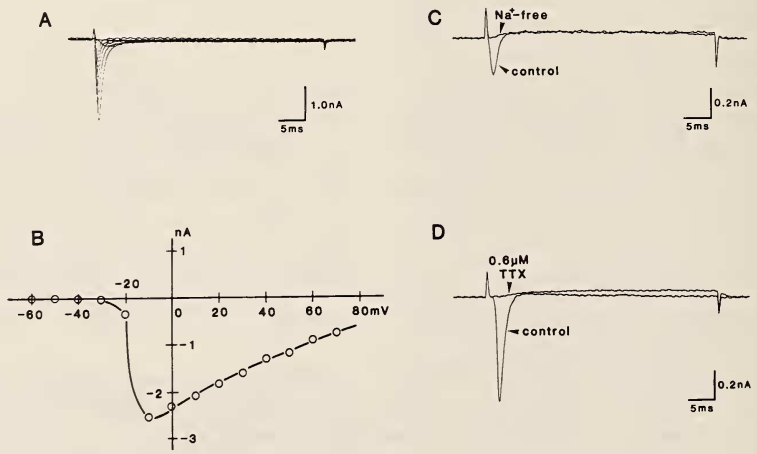

Fig. 2. Na^+ current. A, transient inward currents evoked in a taste cell recorded after removal of outward currents by substitution of Cs^+ for K^+ in the pipette. The taste cell was stimulated by 40 ms-voltage steps between −40 and 80 mV in 20 mV increment from a holding potential of −60 mV. B, I-V relationship for peak inward current from the same cell in A. Holding potential was −60 mV. C, effect of removal of Na^+ on the inward current of another taste cell. Superimposed recording of responses to depolarizing voltage steps to −20 mV from a holding potential of −60 mV in normal saline and Na^+-free saline. D, effect of TTX on the inward current of a third taste cell. Superimposed recording of responses to depolarizing voltage steps to 0 mV from a holding potential of −60 mV before and after addition of 0.6 μM TTX. The pipette contained K-Mg-ATP sol. in C and D.

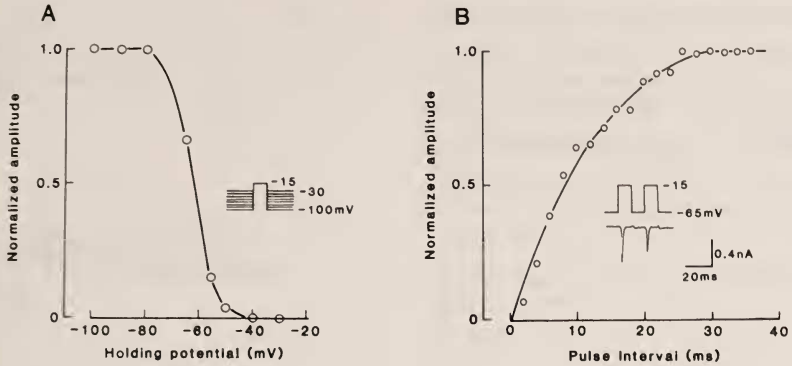

FIG. 3. Inactivation and its recovery of the Na^+ current. A, a steady state inactivation curve for the Na^+ current. Half inactivation was at -57 mV and complete inactivation occurred at -40 mV in this taste cell. B, recovery from inactivation of Na^+ current in the same cell in A. The recovery time constant was 12.5 ms in this taste cell. The pipette contained Cs-Mg-ATP sol.

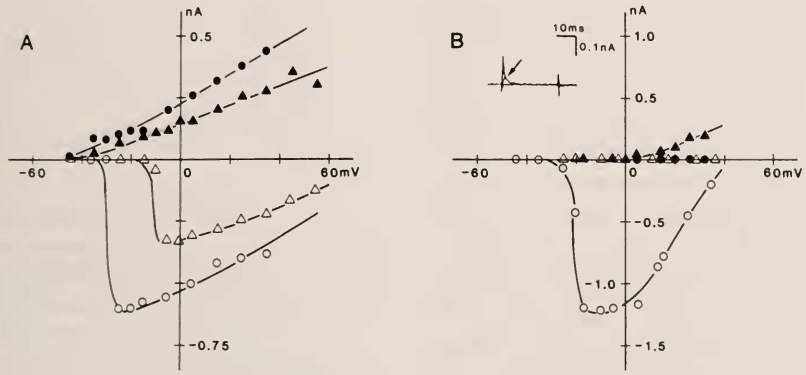

FIG. 4. Effects of Co^{2+} on inward and outward currents. A, inhibitory effect of Co^{2+} on voltage-dependent currents. The I-V relationships show peak inward (○, △) and steady state outward (●, ▲) currents in normal saline (○, ●) and saline containing 10 mM Co^{2+} (△, ▲) in the bath. Holding potential was -65 mV, and the pipette contained K-Mg-ATP sol. B, complete blocking of Na^+ and K^+ currents in another taste cell. The I-V relationships show a peak inward current (○, △), and a transient (▲) and a steady state (●) outward current before (○, ●) and during (△, ▲) perfusion with saline containing 77 mM Ba^{2+} substituted for Na^+ and K^+ (high Ba^{2+} saline). Currents records during perfusion with high Ba^{2+} saline are shown as inset. Holding potential was -65 mV.

represents the recovery of inactivated inward current when a pair of 10 msec (ms) voltage steps from -65 to -15 mV (see inset) was applied at various intervals. The recovery time constant from inactivation was 9.7 ± 2.1 ms ($n=3$). All these properties suggest that the early inward current passes through TTX-sensitive, voltage-gated Na^+ channels.

Lack of Ca^{2+} current

The presence of Ca^{2+} current in a bullfrog taste cell has been inferred from the results of intracellular recordings [4]. When 10 mM Co^{2+} was added to the bath, inward and outward currents were suppressed (Fig. 4A). The decrease in peak inward current may have been partially due to the reduction in concentration of Na^+ since 10 mM Co^{2+} was substituted for Na^+. Substitution of 1.8 mM Co^{2+} for Ca^{2+} altered the threshold and time course of the action potential, but did not change its amplitude (Fig. 1C). To maximize any voltage-dependent Ca^{2+} currents, both K^+ and Na^+ cur-

rents were blocked by using the Cs-Mg-ATP solution in the pipette and replacing Na^+ with an equivalent amount of Ba^{2+} in the bathing solution (Fig. 4B). Nearly all the K^+ outward currents were blocked by the Cs-Mg-ATP solution (△), and the Na^+ inward currents (○) were completely blocked by perfusion with high Ba^{2+} saline (●). In Fig. 4B, a small transient outward current (arrow in the inset and ▲) appeared with voltage steps to potential positive to −5 mV. This current might be carried by Na^+ through Na^+ channels since the pipette solution containind 12 mM Na^+. No convincing evidence for voltage-dependent Ca^{2+} currents was obtained in bullfrog taste cells under the condition of the present experiment.

K^+ current

The outward current was carried by K^+ through K^+ channels because it was blocked by high Cs^+ in the pipette and high Ba^{2+} in the bath (Fig. 4B). The outward current was suppressed by 1–10 mM 4-AP, but was not by 20 mM TEA. Poor sensitivity of K^+ currents to TEA also has been reported in another species of frog, *R. ridibunda* [9]. The magnitude, time course and sensitivity to drugs of the outward currents varied from cell to cell. 15% of the taste cells examined produced only a small outward current, preceded by a large transient inward current in response to a voltage step to 20 mV from a holding potential of −65 mV [17].

1) *Ca^{2+}-dependent K^+ current*

The amplitudes of the outwasrd currents were also suppressed by 10 mM Co^{2+} (Fig. 4A) over the entire voltage range of activation. However, it was not clear whether or not the threshold of activation changed during the suppression. Suppression of the outward K^+ current was also observed when Ca^{2+} was removed from the bathing solution (Fig. 5). In Figs. 5A and B, currents to depolarizing voltage steps were evoked in normal (A), and Na^+-free and Ca^{2+}-free saline solution (B), respectively. The difference between currents in A and B is shown in Fig. 5C. In Fig. 5D, the difference between outward currents alone in A and B is plotted against command voltages from a holding potential of −60 mV. It is seen that the residual outward currents were strongly activated at the

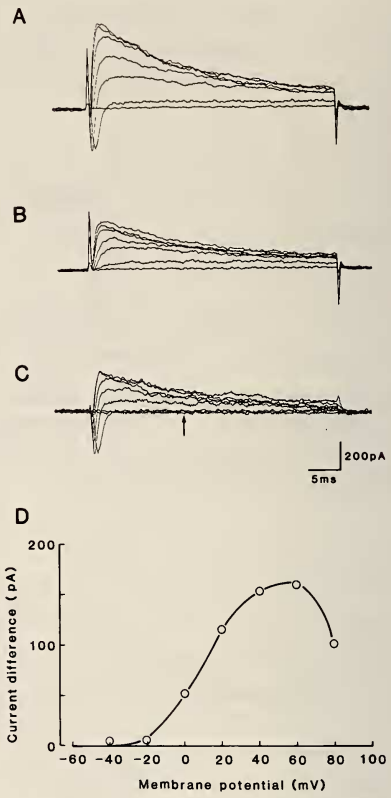

Fig. 5. An evidence of Ca^{2+}-dependent K^+ current. A, currents evoked by 40 ms-voltage steps between −40 and 80 mV in 20 mV increment from a holding potential of −60 mV in normal saline. B, currents of the same cell in A in Ca^{2+}-free and Na^+-free saline. C, difference of currents between A and B. D, difference between the outward currents in normal saline, and Ca^{2+}-free and Na^+-free saline is plotted against the membrane potential. The current difference was measured 15 ms after onset of voltage steps (an arrow in C). The pipette contained K-Mg sol.

range from −20 to 60 mV and had a peak at approximately 50 mV. Similar current difference was found by adding 1.8 mM Co^{2+} to the bath (not shown). Therefore these current differences are due to Ca^{2+}-dependent K^+ currents.

2) *Transient K^+ current*

Outward K^+ currents were divided into two components by difference of time course: a transient component and a sustained component (Figs. 1, 5). These two components appeared even in a Ca^{2+}-free saline solution (Fig. 5B). Figure 6A

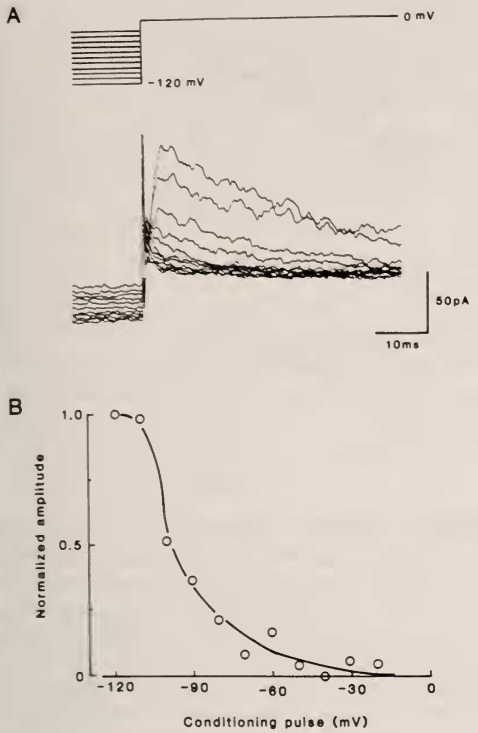

FIG. 6. Transient outward K^+ current activated after strong hyperpolarization. A, recordings of transient outward currents induced after 40 ms-hyperpolarizing conditioning pulses between −20 and −120 mV in 10 mV increment. B, steady-state inactivation curve for the outward current from the same cell in A. Peak currents were plotted against conditioning pulses after subtraction of leakage and capacitive transient currents. Holding potential was 0 mV, and the pipette contained K-Mg sol.

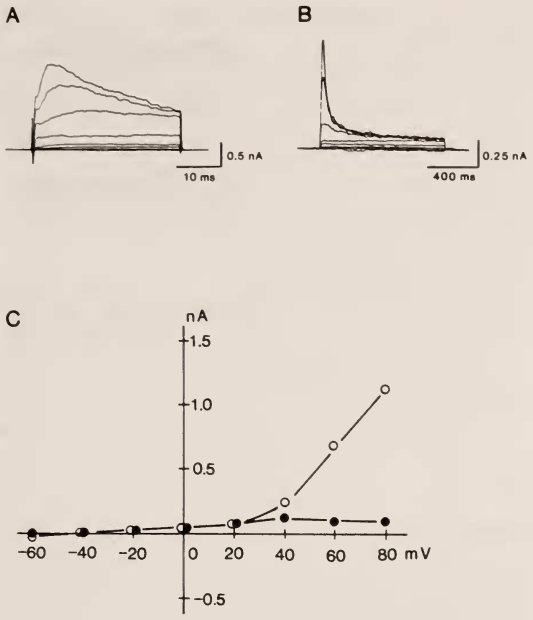

FIG. 7. Transient and sustained components of Ca^{2+}-independent outward currents. A, outward currents induced by 30 ms-voltage steps between −60 and 80 mV in 20 mV increment from a holding potential of −40 mV. B, outward currents induced by 1 s-voltage steps between −60 and 80 mV in 20 mV increment from a holding potential of −40 mV from the same cell in A. C, I-V relationships of transient (○) and sustained (●) components of outward currents obtained from the same recording in B. Holding potential was −40 mV. The bathing solution was a Na^+-free saline solution containing 2 mM Co^{2+} and the pipette solution contained K-Mg sol.

shows outward currents which were activated transiently by voltage steps to 0 mV when the voltage steps were preceded by strong hyperpolarizing conditioning pulses. More than 90% of the transient outward currents was inactivated at potentials positive to −40 mV (Fig. 6B). The activation of transient outward currents started at the potentials between −40 and −20 mV when the holding potential was −60 mV. The transient outward currents were reduced in high K^+ (52.5 mM) saline solution.

Even when the holding potential was more positive than −40 mV, the transient component of outward currents was still activated. An example is shown in Fig. 7 where the taste cell was bathed in a Na^+-free solution containing 2 mM Co^{2+}. Because this concentration of Co^{2+} seems to block Ca^{2+}-dependent K^+ current completely, the outward currents shown in Fig. 7 would not contain Ca^{2+}-dependent K^+ currents. Recordings obtained from the same cell in different time scales are shown in Fig. 7A and B. The transient outward current (○ in Fig. 7C) was greatly activated by voltage steps to potentials more positive than 40 mV from a holding potential of −40 mV.

3) *Sustained K^+ current*

When the membrane potential was held at −40 mV, both transient and sustained components of outward currents evoked by voltage steps between

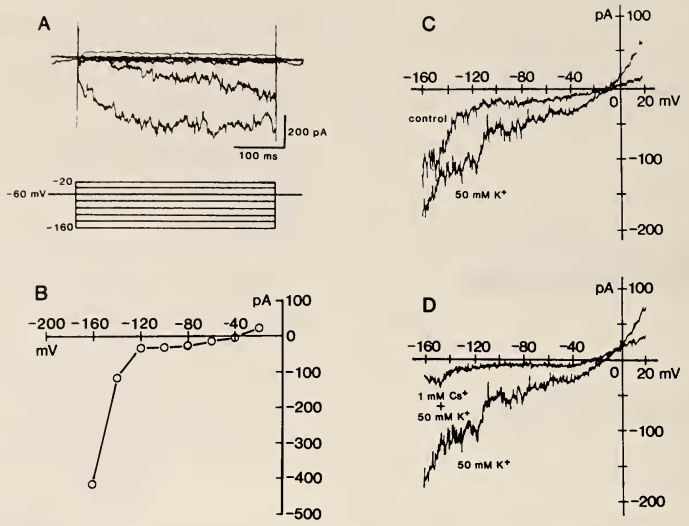

FIG. 8. Evidence for inward rectifier currents. A, currents induced by 400 ms-depolarizing and hyperpolarizing voltage steps between −20 and −160 mV from a holding potential of −60 mV in normal saline. B, steady state I-V relationships of inward currents obtained from the same cell in A in normal saline. Steady state inward currents were measured immediately before the end of voltage steps. C, inward rectifier currents evoked by the falling phase of voltage ramps, in which the membrane potential was changed from 20 to −160 mV at a constant rate of 0.1 V/s, in normal saline (control) and saline containing 50 mM K^+ substituted for Na^+ (50 mM K^+). The recording was obtained from a different cell. D, suppression of the inward rectifier current evoked by the voltage ramp in 50 mM K^+ saline (50 mM K^+) by 1 mM Cs^+ (1 mM Cs^+ + 50 mM K^+). The recording was obtained from the same cell in C. The pipette contained K sol. The bathing solutions contained 1 μM TTX.

−40 and 20 mV were markedly reduced. Although the transient component was inactivated within 200 ms, the sustained component was not inactivated at all even 1 sec(s) after onset of voltage steps (Fig. 7B). The inactivation of the sustained current was not seen at least 5 min after the holding potential was shifted from −60 to −40 mV. The amplitude of the sustained outward currents peaked at 40 mV (● in Fig. 7C).

4) *Inward-rectifier K^+ current*

Figure 8A shows inward rectifier K^+ current induced by depolarizing and hyperporarizing voltage steps from a holding potential of −60 mV in normal saline. Figure 8B shows steady state I-V relationship obtained from the same recordings in A. Hyperpolarization-induced sustained inward current in the bullfrog taste cell in normal saline remarkable appeared at the potentials more negative than −100 mV close value to the equilibrium potential of K^+ (−93 mM). Figure 8C shows inward rectifier currents in normal saline (control) and in saline containing 50 mM K^+ (50 mM K^+). The inward currents were evoked by the falling phase of a voltage ramp in which the membrane potential was changed form 20 to −160 mV at a constant rate of 0.1 V/s. In 50 mM K^+ saline, a larger inward current was induced even at potentials nuch more positive than −100 mV. This current was markedly suppressed by 1 mM Cs^+, a potent blocker of the inward rectifier K^+ channel [21], added to the bathing solution (1 mM Cs^+ + 50 mM K^+, Fig. 8D). In Fig. 8C, the reversal potential in normal saline is seemingly the same as that in 50 mM K^+ saline. This is probably due to simultaneous activation of the sustained K^+ current. These results suggest that the inward rectifier currents evoked by hyperpolarizing voltage steps or voltage ramp were primarily carried by K^+.

DISCUSSION

We have established a dissociation method for bullfrog taste cells and demonstrated that taste cells are identifiable by their characteristic morphology. Taste cells typically have, on their apical

ends, three dendritic branches, which are connected to each other by a thin membranous structure. The morphology of the isolated cells is consistent with that reported in previous ultrastructural studies [1, 2], and in recent work with the dye injection in our laboratory [20]. We observed bipolar cells without dendritic branches of the apical process after vigorous trituration during dissociation process. The electrophysiological properties of these cells were approximately the same as those of taste cells. Thus, we concluded that the bipolar cells without dendritic branches observed in the bullfrog in the present experiment probably are taste cells which have lost branches of the apical process during dissociation.

A single action potential was observed in 85% of the bullfrog frog taste cells in response to depolarization of 20 mV from the resting potential (−75 mV in average) [17]. This suggests that bullfrog taste cells should be able to generate action potentials *in situ*, if their resting potentials are as large as those in isolated cells. However, *in situ* taste cells in the bullfrog usually have resting potentials between −20 and −30 mV in the average and do not discharge action potentials when the tongue surface is adapted to normal saline [3, 12, 14, 15, 22], whereas *in situ* taste cells of mudpuppy produced action potentials with large resting potentials [6–8]. Recently Sata & Sato (1988) [20] found dye-coupling between supporting cells and some taste cells. In general, supporting cells within the taste disc [3, 13, 22] or epithelial cells surrounding the taste buds [7, 11] have low resting potentials. However, in the catfish [23] and the mudpuppy [24], taste buds cells were not dye-coupled to epithelial cells surrounding the taste buds. Thus, it is possible that the resting potentials of frog taste cells which are electrically coupled to supporting cells are significantly lower than those of isolated taste cells. Consequently, these cells might be unable to generate any action potential *in situ* because most of the voltage-dependent Na^+ channels are inactivated at the resting potential *in situ* (Fig. 3A).

We have never observed repetitive spike generation in isolated taste cells even with a long-lasting depolarization. This result is similar to that obtained from the taste cells in another species of the frog, *R. ridibunda* but different from that obtained from the taste cells in tiger salamander [11]. In the tiger salamander taste cells, a train of 2–5 action potentials was observed in response to sustained depolarizing currents [11]. The difference may be partially attributed to difference in the recovery time from inactivation of the Na^+ current [9, 11]. In fact, the time constant of recovery of Na^+ currents in the bullfrog taste cell (Fig. 3B) was twice as longer as that in the bullfrog sciatic nerve [21].

We could not find any voltage-dependent Ca^{2+} current in the bullfrog taste cells (Fig. 4B) although there is an indication of the presence of Ca^{2+}-dependent K^+ channel (Fig. 5). With the intracellular recording method, it has been reported that anodal break action potentials were suppressed by 10 mM Co^{2+} [4]. We also observed suppression of a transient inward current by 10 mM Co^{2+} (Fig. 4A). However, 1.8 mM Co^{2+} raised the threshold for the inward current without reduction of the amplitude (Fig. 1C). In addition, 10 mM Co^{2+} inhibited the steady state K^+ outward current over the whole range of activation so that the I-V relation curve shifted in depolarizing direction as a whole (Fig. 4A). In *R. ridibunda*, 5 mM Ni^{2+} showed a similar effect, which was attributed to a screening effect of negative surface charges [9]. Since there are synaptic connections between taste cells and gustatory nerve fibres [1, 2], Ca^{2+} influx activated by cell depolarization is presumed to exist. However, we could not detect it under our recording condition probably due to the samll amplitude or the rapid run-down of Ca^{2+} currents.

Ordinary K^+ currents other than Ca^{2+}-dependent K^+ currents which contribute to generating of outward currents were classified into two components: a transient component and a sustained component. Most of the transient currents by depolarizing voltage steps were inactivated by preceding conditioning pulses positive to −40 mV, and the activation started at the same potential range as the threshold of TTX-sensitive Na^+ currents (Fig. 6). These properties suggest that the transient component of outward currents is a type of A currents [21]. However, when the membrane potential of the taste cell was held at −40 mV, the

transient currents were still activated by strong depolarizing voltage steps to potentials more positive than 40 mV (Fig. 7), but greatly reduced at the range of −40 to 20 mV where both components of outward currents were certainly activated when the membrane potential was held at potentials negative to −60 mV (Fig. 4A). Therefore, it remains undetermined whether or not the transient K^+ currents activated by depolarizing voltage steps following strong hyperpolarizing conditioning pulses are the same as those activated by strong depolarizing voltage steps from holding potentials positive to the resting membrane potential.

A sustained component of outward currents was observed following inactivation of the transient component within 200 ms after onset of voltage steps. This current was obtained even when the membrane potential was held at −40 mV (Fig. 7). These properties are consistent with those of a delayed rectifier K^+ current. The sustained K^+ current in bullfrog taste cells was not inactivated at least 5 min after voltage shift from −60 to −40 mV, resulting in a continuous decrease of membrane resistance. This may be another reason why repetitive spike generation is never evoked in isolated bullfrog taste cells. Since the amplitude of the sustained outward current peaked at 40 mV (Fig. 7C), the current component is distinguished from membrane leakage current.

We were unable to discriminate the trasient component from the sustained component pharmacologically because both components were blocked by 1–10 mM 4-AP but not blocked by 20 mM TEA. In addition, the amplitude and the time course of outward currents displayed a great diversity from taste cell to taste cell. Recently, it has been suggested in mammalian brain that there are several K^+ channel forming proteins which derive from a family of genes but have different functional properties, and that the diversity of voltage-gated K^+ channels depends on degree of assembly of those K^+ channel forming proteins to K^+ channels [25]. Similar mechanism may underlie the diversity of K^+ channel properties in bullfrog taste cells. It this is the case, it probably is difficult to divide K^+ channels into subtypes with pharmacological or electrophysiological method.

In this experiment, an inward rectifier current was observed in normal and high K^+ saline (Fig. 8). Our results indicate that the amplitude of inward rectifier current and the reversal potential seemed to change depending upon the external K^+ concentration, and the currents were markedly reduced by Cs^+, a potent blocker of inward rectifier K^+ channel. Therefore, we concluded that inward-rectifier K^+ channels exist in bullfrog taste cells, while they have not been reported in taste cells of another species of the frog, *R. ridibunda* [9].

This type of channel has also been reported in the mudpuppy taste cells [10], as well as the other sensory cells [26, 27]. Inward rectifier channels seem to play a particularly important role in the taste cell. The apical surface of the taste cell is exposed to somewhat variable conditions, such as a high concentration of salts and tap water. When various hypotonic solutions are applied to the apical surface of taste cells, the inward rectifier channels could prevent the taste cell from excessive hyperpolarization. This results in holding an appropriate range of the membrane potential to maintain sensitivity of taste cells. Furthermore, these channels could increase the sentitivity of the taste cell to low concentrations of potassium salt stimuli because the I-V relationship shifts depending on the external K^+ concentration [21].

Thus, if the inward rectifier K^+ channels locate in the receptive membrane of the taste cells, the inward current produced by the inward rectifier K^+ channel as well as other K^+ channels can contribute to gestatory transduction of KCl stimuli into the receptor potential as suggested in mudpuppy taste cells [28].

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REFERENCES

1 DeHan, R. S. and Graziadei, P. R. C. (1971) Functional anatomy of frog's taste organ. Experientia, **27**: 823–826.

2 Graziadei, P. P. C. and DeHan, R. S. (1971) The ultrastructure of frog's teste organ. Acta Anato., **80**: 563–603.

3 Sato, T. (1980) Recent advances in the physiology of taste cells. Prog. Neurobiol., **14**: 25–67.

4 Kashiwayanagi, M., Miyake, M. and Kurihara, K. (1983) Voltage-dependent Ca^{2+} channel and Na^{+} channel in frog taste cell. Am. J. Physiol., **244**: C82–C88.

5 Teeter, J. H., Funakoshi, M., Kurihara, K., Roper, S., Sato, T. and Tonosaki, T. (1987) Generation of the taste cell potential. Chem. Senses, **12**: 217–234.

6 Roper, S. D. (1983) Regenerative impulses in taste cells. Science, **220**: 1311–1312.

7 Kinnamon, S. C. and Roper, S. D. (1987) Passive and active membrane properties of mudpuppy taste receptor cells. J. Physiol., **383**: 601–614.

8 Avenet, P. and Lindemann, B. (1987) Action potentials in epithelial taste cells induced by mucosal Ca. J. Membr. Biol., **95**: 265–269.

9 Avenet, P. and Lindemann, B. (1987) Patch-clamp study of isolated receptor cells of the frog. J. Membr. Biol., **97**: 223–240.

10 Kinnamon, S. C. and Roper, S. D. (1988) Membrane properties of isolated mudpuppy taste cells. J. Gen. Physiol., **91**: 351–371.

11 Sugimoto, K. and Teeter, J. H. (1990) Voltage-dependent ionic currents in taste receptor cells of the larval tiger salamander. J. Gen. Physiol., **96**: 809–834.

12 Sato, T. and Beidler, L. M. (1975) Membrane resistance change of the frog taste cells in response to water and NaCl. J. Gen. Physiol., **66**: 735–763.

13 Akaike, N. and Sato, T. (1976) Role of anions and cations in frog taste cell stimulation. Comp. Biochem. Physiol., **55A**: 383–391.

14 Akaike, N., Noma, A. and Sato, M. (1976) Electrical responses of frog taste cells to chemical stimuli. J. Physiol., **254**: 87–107.

15 Miyamoto, T., Okada, Y. and Sato, T. (1988) Ionic basis of receptor potential of frog taste cells induced by acid stimuli. J. Physiol., **405**: 600–711.

16 Miyamoto, T., Okada, Y. and Sato, T. (1989) Ionic basis of salt-induced receptor potential in frog taste cells. Comp. Biochem. Physiol., **94A**: 591–595.

17 Miyamoto, T., Okada, Y. and Sato, T. (1988) Membrane properties of isolated frog taste cells: three types of responsiveity to electrical stimulation. Brain Res., **449**: 369–372.

18 Armstrong, D. and Eckert, R. (1987) Voltage-activated calcium channels that must be phosphorylated to respond to membrane depolarization. Proc. Natl. Acad. Sci. USA, **84**: 2518–2522.

19 Hamill, O. P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F. J. (1981) Improved patch-clamp techniques for high-solution current recording from cells and cell-free membrane patches. Pflügers Arch., **391**: 85–100.

20 Sata, O. and Sato, T. (1988) Dye-coupling among cells in taste disk of frog. Proc. Jpn. Symp. Taste Smell, **22**: 157–160.

21 Hille, B. (1984) Ionic channels of excitable membranes. Sinauer Associates Inc., Sutherland.

22 Sata, O. and Sato, T. (1989) Electrical responses of supporting taste cells in the frog taste organ to chemical stimuli. Comp. Biochem. Physiol., **95A**: 115–120.

23 Teeter, J. H. (1985) Dye-coupling in catfish taste buds. Proc. Jpn. Symp. Taste Smell. **19**: 29–33.

24 Yang, J. and Roper, S. D. (1987) Dye-coupling in taste buds in the mudpuppy, *Necturus maculosus*. J. Neurosci., **7**: 3561–3565.

25 Stümer, W., Ruppersberg, J. P., Schröter, K. H., Sakmann, B., Stocker, M., Giese, K. P., Perschke, A., Baumann, A. and Pong, O. (1989) Molecular basis of functional diversity of voltage-gated potassium channels in mammalian brain. EMBO J. **8**: 3235–3244.

26 Ohmori, H. (1984) Studies of ionic currents in the isolated vestibular hair cell of the chick. J. Physiol. **350**: 561–581.

27 Badar, C. R., Bertrand, D. and Schwartz, E. A. (1982) Voltage-activated and calcium activated current studied in solitary rod inner segments from the tiger salamander. J. Physiol., **331**: 253–284.

28 Kinnamon, S. C. and Roper, S. D. (1988) Evidence for a role of voltage-sensitive apical K^{+} channels in sour and salt taste transduction. Chem. Senses **13**: 115–121.